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The Histological Structure of the Gills of the Najades with Special Reference to the Histology of the Groove Along the Edge of the Inner Gill.

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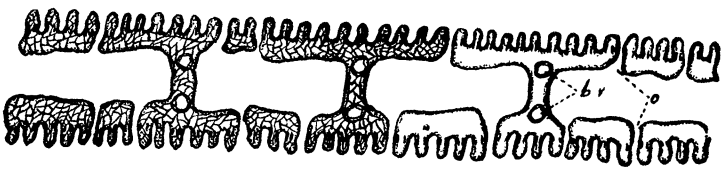
I.—INTRODUCTORY.

The primary purpose of the investigation here recorded has been to determine the histological structure of the gill of the Najades with special reference to the groove along the ventral edge of the inner gill. It was at first intended to investigate the groove with the view of ascertaining its function. After some work was done along this line it was found that Allen in a recent paper (2) (1921) had investigated the problem very thoroughly and arrived at conclusions which the present work corroborated in so far as it had been completed, namely that the function of the groove is to act as a channel for conveying food to the labial palps. The first mention of this problem so far as the author is aware is by Dr. Arnold E. Ortmann of the staff of the Carnegie Museum in his Monograph of the Najades of Pennsylvania (4). On page 294 of that publication he says, "Making cross sections through the gills we observe that the outer edge of the outer gill is simply rounded off while in the inner gill a peculiar longitudinal furrow extends along its edge, which may be seen microscopically. As to the meaning of the furrow, I can not make any suggestion and am only able to state the fact of its presence. By the presence of this furrow an inner may always be distinguished from an outer gill."

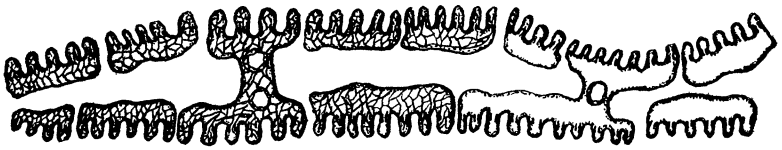
PLATE I

Figure I



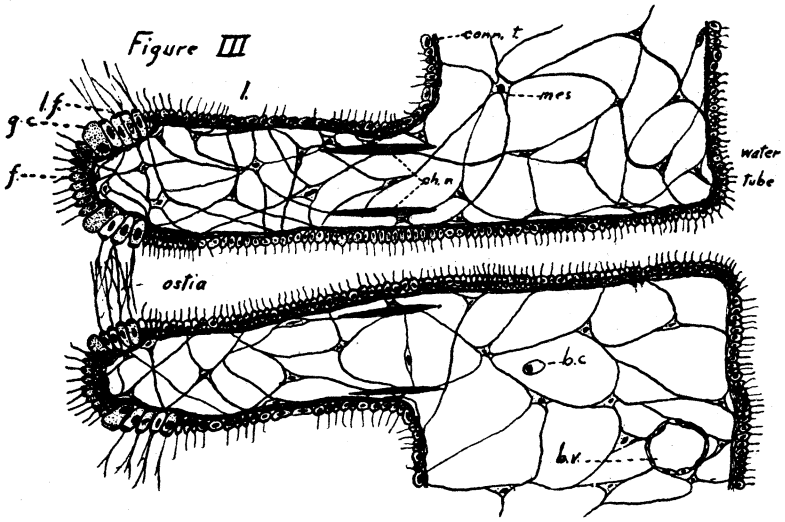
Transverse section through outer gill
Lampsilis siliquoidea – male

Figure II

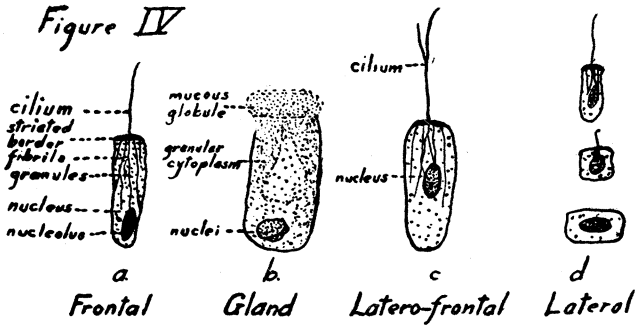


Transverse section through inner gill.
Amblema peruviana – female

PLATE II



Transverse section through ostia and filaments



Types of different epithelial cells of the gill

Peck in his "Study of the Lamellibranch Gill" (Quart. Jour. Mic. Sci.) (5) investigated the histological structure of the gills with the object of ascertaining the morphological equivalent of the gill tentacles of other more primitive Pelecypoda. As his work was entirely on marine species of course his work is without mention of the furrow which is the subject of this paper. The author however has found as would be expected, many points of similarity between the histological structure of the gills of the Najades and that of the forms investigated by Peck. Points of similarity will be mentioned later.

The material used in the investigation was entirely from the Mississippi drainage system and consisted of the following species (nomenclature after Ortmann): Lampsilis siliquoidea (Barnes), Anodonta corpulenta (Cooper), Amblema peruviana (Lam.), Elliptica dilitatus (Raf.), Lasmigona complanata (Barnes), Obliquaria reflexa (Raf.), Fusconaja undata (Barnes), Proptera alata (Say), Lampsilis fallaciosa (Smith).

II.—METHOD OF INVESTIGATION.

About fifty different specimens of various ages, and ones representing the different species named in the introduction furnished the material for this study. Such a selection was necessary because Ortmann has shown that in different species there is considerable variation in the structure of the gill especially during the marsupial period (4), and it was desired that the study of the histological structure should be as general as possible with the material at hand.

In every case live mussels were used and when opened the gills were cut out, placed in the different fixatives, and labelled according to name, sex, left or right, inner or outer, gill. In a few specimens the sex as judged from the apearance of the shell was on microscopical examination found to be erroneous and the data changed accordingly. The septa in the female separating the water-tubes in the marsupial gill are much more crowded than in the male, and in the final decision as to sex this point was given preference over shape of the

shell. Ortmann has used this method when a microscopical examination was made.

The fixatives used were Petrunkevitch, corrosive sublimate with glacial acetic acid, Perenyi, Cox-Golgi, and Zenker. Of these the best results were obtained with the first three, Zenker's fluid was entirely unsatisfactory and the Cox-Golgi was almost as bad. Both showed a decided tendency to distort the gill, and were very hard to remove in washing. Only one gill prepared in each of these two fixatives was sectioned and the results were so unsatisfactory that the sections were discarded without an attempt being made to study them. The Petrunkevitch fluid was found to be the most satisfactory in every way. The gills were washed in tap water for twenty-four hours, and in the case of the corrosive acetic until the iodine test showed that excess fixative had been removed. The material fixed in Zenker's fluid was washed in semi-darkness in an unsuccessful attempt to avoid precipitation.

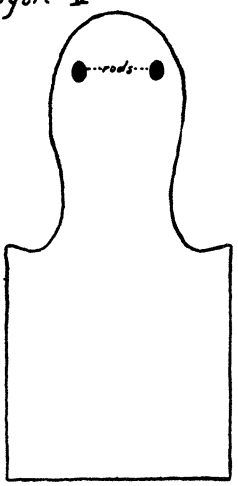
The Heidenhain, Ehrlich and Delafield haematoxylins were found to be the most satisfactory stains. All gave very good results and there was little to choose from among them. Mayer's haem-alum was also used but usually the stain of the necleus with it was too faint.

The material was stained in bulk for twenty-four hours, being taken from 70% alcohol into the stain; it was then taken back into 70% alcohol to avoid any distortion due to possible diffusion currents and then carried through the alcohols into xylol, remaining twenty-four hours in each. After the material had been in xylol twelve hours, melted paraffin was slowly added until the limit of solubility of the paraffin was reached. In this way the paraffin was slowly infiltrated into the tissue at the same time that clearing was taking place. The vials containing the gills were then placed on a warm plate until most of the xylol was evaporated, and then the tissues were transferred to the melted paraffin of the imbedding oven and imbedded in the usual way. By this method of paraffin infiltration ali sudden changes of temperature were avoided and the concentration of the paraffin in the tissues very gradually increased as the xylol evaporated.

Serial sections were used throughout the investigation, the

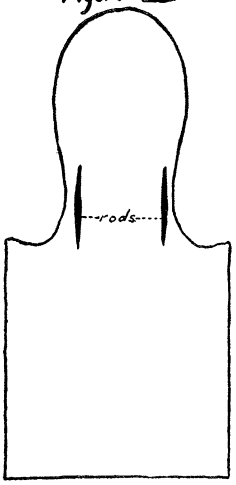
PLATE III

Figure V



Chitinous rods as they appear in marine Polycopoda
After Peck

Figure VI



Chitinous rods as they appear in the Najades

Diagrams to show the difference in position and form of the chitinous rods in the gill filaments of marine and fresh-water Lamellibranchs

thickness was varied from 3.3 m. to 20 m. but those from 6.6 m. were most satisfactory and this thickness was used in most of the work. After fixing to the slides the sections were de-colorized by 70% acid alcohol in a Petri dish under observation with the microscope.. When the decolorization had proceeded far enough the slides were transferred to distilled water to which had been added a drop of ammonium hydroxide solution to neutralize the action of the acid during the time taken in transferring the slides from the acid alcohol to the water. After washing in this way the stain gave excellent results. The slides were then carried up through the alcohols and xylol and covered with balsam and cover glass in the usual manner. This method of decolorizing sections stained in bulk with haematoxylin and washing in distilled water to which a trace of alkali has been added, was found to be much more satisfactory than the use of ordinary tap weter since the sections were entirely free fro mdirt.

The sections were cut in the three different planes of the gill, that is vertical, longitudinal-lateral, and longitudinal-transverse, the longitudinal-lateral section was only possible with small gills and even then was not very satisfactory since the sections were so large that complete serial sections were difficult to obtain.

III.—CONCLUSIONS AND RESULTS.

The simplest structure of the gills as was previously noted by Ortmann (4) is always found in the gills of the male. Figure 1 is a cross section of an outer male gill and shows the comparatively few filaments or septa between the interlamellar junctions. In figure 2 an innner female gill is shown and the greater distance between the inter-lamellar junctions, as measured by the number of septa between them, is the striking thing noticed. The outer lamella of the inner gill is also convex instead of being parallel to the inner lamella as it is in the other gill. The effect of these differences between the male and femal gills is to give a much greater size to the water tubes in the latter which is of course of great value during the period of incubation of the glochidia.

The sub-filamentar outgrowths are large and abundant so

as to completely mask the primary tubular character of the gill filaments. These outgrowths at the bases of the filaments which bind the filaments together into the lamella or plate, are broken at irregular intervals (o-fig. 1 and 2) giving rise at these interruptions to the ostia or water openings into the water tubes (w. t. fig. 1 and 2).

Figure 3 shows a single lamella highly magnified. The sketch shows the lamella to be covered with ciliated columnar epithelium of which three kinds may be distinguished. These may be called from their positions on the filament, frontal (f), latero frontal (l.f.), and lateral (l).

The cells of the frontal epithelium are ordinary columnar epithelium averaging 3 microns in length. They rest on a continuous base of connective tissue which extends along the lamella bending into each filament, and is only interrupted at the ostia. The cytoplasm of these cells is very granular especially near the marginal end while the nucleus is at the base of the cell or better, the distal end. The nucleus in a few of the cells is found near the middle of the cell. Some of the cells are without doubt mucous gland cells and occasionally one may be seen of the characteristic goblet cell appearance which has discharged its secretion of mucous. From the appearance of these epithelial cells it would appear that any of them can function either as an epitheleal ciliated cell or a glandular cell. The cilia borne on these cells vary in length from one to two microns. The cells are on the average one micron or less in diameter giving a ratio of length to width of 3 : 1.

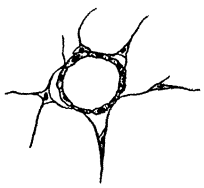
Adjacent to the frontal epithelium on either side of the lamella is found the lateral-frontal epithelium. The distinguishing character of this tissue is the remarkable length of the cilia and the comparative width of the cells. So long are these cilia that they bridge the space between the filaments and are entangled with those from the opposite cells of latero-frontal epithelium and for this reason the length of these cilia must be estimated rather than measured. Their estimated length is about four microns. These cells are very scant compared to the number of the others; usually only four or five cells occur on each side of the filament, one filament was found

PLATE IV

Figure VII

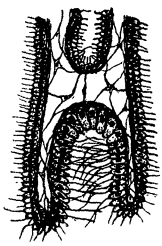
Longitudinal cross-section through anterior end of gill showing the long cilia in the groove - Drawn with Edinger apparatus

Figure VIII



Structure of blood vessel from interlamellar junction showing attachment of supporting cells

Figure IX



Note:- Depth of groove much reduced compared to size of cells

Vertical section through groove

bearing eight cells on each side, counts of three were frequent. Usually this group of cells is separated from the frontal epithelium by a single gland cell which was usually very large, ? microns wide by five microns long. The mucous globule full of granules was always found extruded from the top of this cell probably due to fixing. The cells of the latero-frontal epithelium were columnar in shape but were larger than those of the frontal epithelium. Their average length was four microns, while the width varied from one to two microns being greater in those cells nearest the distal end. This variation in the thickness of these cells is probably due to less crowding of these nearest the inter-filamentar junction since the proximal cells are on the curve of the filament (see fig. 3). The cytoplasm of these cells is not so granular as that of the frontal epithelium. The nucleus has the same ellipsoid shape as in the frontal epithelium but is slightly larger and located nearer the center of the cell. The neuclolus is always very distinct in these cells.

The epithelial cells from this point gradually assume a more spherical shape and although bearing cilia, the cilia are short, usually less than one micron in length. About one-half the distance from the edge of the filament to the inter-filamentar junction the cells have assumed a spherical shape and the nucleus is also practically spherical at this point. From this point on the cells gradually lengthen in the other plane giving rise to the pavement epithelium lining the ostia and water tubes. The nucleus also gradually lengthen until the ellipsoid form appears again but this time the long axis of the ellipse is parallel to the base instead of perpendicular as before. These cells are from three to four microns long and an average two microns in width. The ratio of length to width is therefore three to two, or rarely four to two, and never becomes as great as that in the frontal or latero-frontal epithelium. It should be remembered however that this ratio is the converse of that mentioned first since the cells are now flattened in the horizontal direction instead of the vertical, that is the former width has by gradations now become the length of the cell and vice versa. Not all of these pavement cells retain their cilia but the number is so variable that no stand-

ard can be worked out that will hold true for the whole gill. However almost all the cells lining the ostia and the water tubes retain their cilia and are usually spherical in shape. Some even retain the columnar form and they are never flattened horizontally like those lining the inter-lamellar junctions.

The nuclei of these different types of epithelial cells, though varying in size, in proportion to the size of the cell, are remarkably similar in all other respects so that the following description will hold for all of them. As mentioned before the nucleus is usually ellipsoid in form but is spherical in those cells which may be classified as cubical epithelium. The nuclear membrane is always very distinct and stained heavily with haematoxylin. The neucloplasm is full of dark-staining granules one of which is much larger and more distinct than the others and for this reason is called the nucleolus. The nucleo-plasm as a whole seems to take a darker stain than the cytoplasm but this appearance may be due to the optical effect of the numerous granules.

The nucleus of the single gland cell between the frontal and latero-frontal epithelium (fig. 4) is very large corresponding to the size of the cell, and at times has the appearence of two or even three nuclei clumped together (fig. 4-b.). When it has this form the nuclear membranes are distinct but touch one another. These cells are therefore sometimes multinuclear.

The cells bearing cilia have a striated border at the top to which the cilia are attached. This border is connected with the nucleus by a number of very fine fibrilis which run through the cell protoplasm giving it also a sort of striated appearance. Whether or not these fibrilis are continuous into the cilia, I was unable to ascertain.

The chitinous rods supporting the gill filaments of the Lamellibranches are well known, and their structure was thoroughly worked out by Peck (5). I have found that in the Najades the structure is similar to the marine species used by Peck in that the rods are two in number, one occurring on each side of the filament and similar to his in that they are not continuous througout the lamella but are composed of a

number of short rods overlapping at the ends. This construction gives the gill far more flexibility than it would have if the rods were a single rigid piece. However in his drawings, Peck places the rods out near the end of the filament while I have found them in the Najades to occur near the base of the filament where it joins the interfilamenter junction. In the structure they also differ; Peck shows them as cylinders (circular in cross-section) while in the Najades they are greatly flattened so as to resemble a knife blade with the edge perpendicular to the surface of the lamella. These differences are shown in figures 5 and 6.

The connective tissue which underlays the ciliated epithelium corresponds to areolar connective tissue. When examined under the microscope it is seen to be made up of very fine, transparent white fibers. It is very scant in thickness, but runs along the entire lamella underneath the epithelium, as well as under the cells lining the water tubes, only being interrupted at the ostia, where the bundle of fibers divides and encircles the opening. The connective tissue is shown in figure 3 (conn. t.).

The tissue filling up the space inside the filaments as well as the interfilamentar junctions is in the form of a reticulum.

The cells are stellate in form and have long branching processes of cytoplasm which interlace with each other and even appear to fuse at times with those of other cells. The nuclei of these cells vary from the extreme elliptical to the circular in shape but are usually in the form of an ellipse with the short axis about two-thirds of the length of the long axis. The size of these nuclei varies around one to three microns in diameter. The processes of the cytoplasm weave around one another so much that their length cannot be determined, however I have frequently seen a single strand extending across the entire width of the lamella. The tissue resembles in appearance the mesenchymal cells in primitive mesoblastic tissue. Large blood vessels run through this tissue and some of the smaller vessels open into it. This reticulum thus acts as a blood sinus. The circulation in the gills thus can hardly be called a closed system. Since this reticulum extends out into the filaments the animal is rendered partly independent of

the water tubes for respiration as the filaments themselves can carry on that function though only to a limited extent due to the thickness of the layer of epithelium covering them. The epithelium lining the water tubes is much thinner.

In figure 3 these cells are shown (mes.) with their processes, interweaving with each other. The majority of the cells as is shown in the diagram lie along the connective tissue which forms the base for the epithelium, and send out their branches diagonally across the filament, but many of them are found in the center of the filament supported by their processes running to the sides. Frequently blood corpuscles are found in this reticulum (figure 3 b.c.) between the meshes formed by the strands. Blood vessels of large size are found running vertically through the interlamellar junctions in every gill (figures 1 and 2 b.v.). These blood vessels are composed of cells which are extremely flattened and united at their ends to form the tubes. The cells are curved in an arc, the amount of curvature depending on the number of cells required to encircle the blood vessel. The mesoblastic cells are in turn attached to these cells by their processes or the mesoblast cell may lie against the blood vessels and send out its projections of cytoplasm to others of its kind in the reticulum. This structure insures the blood vessel remaining in the same position guyed as it were by strands of cytoplasm. The structure of a blood vessel with its supports is shown in figure 6.

At the base of the inner gill the filaments making up the lamellae instead of directly doubling back upon themselves as in the outer gill, are thrown upward into a fold which gives the furrow mentioned in the introduction. The furrow was examined in serial sections of entire gills and was found at the extreme anterior end of the gill although it was very shallow at this point since the filaments are so short at the anterior end. It gradually deepened for a distance of about a centimeter along the edge of the gill until the maximum depth was reached which is continued along the gill for the remainder of its length. This depth varies with the size of the animal and to a small extent in different species. The depth of the groove is from one to three millimeters. The maximum depth of three millimeters was only found in one very large

specimen of Amblema peruviana. Figure 7 shows a vertical section through the furrow, drawn with the Edinger projection aparatus. The lamella are seen to fuse at the top of the groove rather than being united by interlamellar junctions. At the point of fusion of the lamella where the groove is discontinued, the cells are much larger than the usual epithelial cells and are closely packed together. The nuclei at this point are spherical (circular in optical section) instead of the ordinary ellipsoid form, probably due to the crowding of the cells. These cells are apparently similar in function and structure to the large gland cell found between the frontal and latero-frontal epithelium on each side of the filament (figure 3 g.c.). However these glands cells in the groove are not mulatinucleated like the ones on the filaments (figure 4 b.), that they have a glandular function is evident from their large size (four microns by five microns), their lack of cilia, and their granular cytoplasm. This function would corresepond with Allen's discovery that food particles imbedded in mucous globules were transported along the groove, since it accounts for the source of the mucous.

The sides of the groove are covered with latero-frontal or rather an epithelium corresponding to it. They are slightly larger than the latero-frontal cells, being five to seven microns long, and three to five microns in width; they both possess the characteristic long cilia, which in this case sometimes reach a length of as great as twenty microns; the measurements of the cilia were difficult to record due to the entangling with those of other cells. These long cilia undoubtedly serve to convey the food and mucous along the groove.

IV.—SUGGESTIONS AS TO THE CAUSES OF THE PHENOMENON AND POSSIBLE SOURCES OF ERROR.

In the different kinds of epithelium of the gill the clue to the selection of food particles and the rejection of mud and sand might be found. Allen has shown that foreign substanced like carborundum, carmine, etc., although introduced into the incurrent siphon are never found in the alimentary tract (Allen 1). He explains this fact by the action of the cilia on the labial palps. The cilia on the gills might help in

this selection, since they must come in contact with these particles before they reach the labial palps. It seems probable that this selection would be made in the groove of the inner gill at least.

The cilia on the gills apparently strain the water entering the gills and probably this is the principal function of the long cilia on the latero-frontal epithelium. These cells are certainly located at a strategic position for this work, situated as they are at the turns of the filaments. The ostia as well as the depressions between the filaments may be compared to streets; these cells might then serve as traffic policemen with their cilia stretched across the opening, permitting the water to enter by turning back dirt and foreign particles.

The method of preparation of the sections, given before, should insure against plasmolysis of the tissues, but it is possible that in some cases this might have taken place. However the uniform results in the tissues investigated makes it very unlikely.

The training of the eye for observation of details is of course an important factor and due to lack of this at the start of the work some important details may have been overlooked. The measurements were taken with standard equipment and it is hardly possible that it was at fault.

The drawings are of course idealized somewhat since all the details of structure would lead to confusion.

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Notes on Alabama Plants.

BY W. WOLF, O. S. B.

A New Monotropoid Plant.

I.

About the first of November, 1901, my attention was drawn to the discovery of a plant by Brother Norbert Knapke, who detected it while he was raking leaves in the forest of the St. Bernard College estate, Cullman County. The man was very enthusiastic about the plant, never before observed by him. He described the plant as leafless, with bell-shaped flowers almost ready to expand. At that time my interest in plant life was very limited. At the man's urgent request, however, I finally consented to accompany him to the habitat of the plant. Having reached the place, he removed the covering viz., fallen leaves of successive seasons, and exhibited a fine group of about a dozen scapes. These scapes were in excellent state and the flower buds far advanced. To all appearence it seemed but a question of a few days or possibly a week until the plant would be in full bloom. Having seen the plant, I was now easily induced to repeat the visit after a few days. In those days, the stock law was not in force, in this section of the state, and this accounts for the loss of the plant. On our next visit we found the whole group of plants up-